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DIETARY SUPPLEMENTATION WITH PHYTOHEMAGGLUTININ IN COMBINATION WITH α-KETOGLUTARATE LIMITS THE EXCRETION OF NITROGEN VIA URINARY TRACT

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Abstract: The aim of the study was to evaluate the effect of both phytohaemagglutinin (PHA) alone, and in combination with alpha-ketoglutaric acid (AKG), on nitrogen elimination via the urinary tract as opposed to the gastrointestinal tract of rats. In experiment 1, rats were assigned to one of two experimental groups, (1) Control and (2) PHA, whilst in experiment 2, rats were assigned to one of three experimental groups, (1) Control, (2) AKG, and (3) AKG+PHA. AKG was administered via drinking water, while PHA was administered via a stomach tube. The stock solution of crude PHA in 0.9% NaCl, was (20% w/v) in water: 50 mg PHA/ml, 20 ml/kg body wt. Rats were 7 weeks old at the start of the experiments. Significantly lower daily weight gains in the AKG+PHA and PHA groups ($p<0.05$) were observed compared to the Control and AKG groups. Increased duodenal crypt depth (138%; $p<0.05$) was noticeable in the AKG+PHA group cf Controls; however, there was no significant difference in the thickness of the tunica mucosa. In the AKG+PHA group, the expression of neuropeptide Y (NPY) in the granula of neuronal cells of the submucosal parasympathetic ganglia was noticeable, although no expression was found in goblet cells. Finally, significant reduction in N excretion in urine was observed in the AKG+PHA, compared with the Control groups ($p<0.05$). It is concluded that a combined PHA and AKG treatment stimulated the small bowel growth via enhanced epithelial turnover, reduced the N excreted in urine and increased the N in faeces.

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Key words: alpha-ketoglutaric acid, phytohemagglutinin, nitrogen elimination, intestine, rat.

INTRODUCTION

Lectins of plant origin are very common in both human and animal diets, with evidence of their interaction with the gut [2, 19]. For instance, oral administration of red kidney bean (*Phaseolus vulgaris*) lectin, phytohaemagglutinin (PHA), over a 3 d period in young suckling rats resulted in gut maturation in terms of epithelial development and decreased lactase as well as increased maltase and sucrase activities [15]. Moreover, the authors reported a reduction in macromolecular absorptive capacity, sometimes referred to as gut closure [15]. Of importance here is the fact that these changes usually occur in rats weaned under normal physiological conditions [26]. PHA also affects the pancreas of rats, leading to an increase in its weight relative to that of body weight (BW) as well as enhancing its exocrine function [15]. It seems logical to accept, therefore, that dietary lectins (including PHA) accelerate the turnover of gastro-intestinal tract (GI-tract) cells. This being the case, rapid cell proliferation might be expected to result in

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both increased gut growth and functional maturation, and as a further consequence, an increase in the use of dietary amino acids for protein synthesis by enterocytes [14, 16].

Glutamine, and one of its derivatives, namely alpha-ketoglutaric acid (AKG), are considered crucial molecules in trans-membrane amino acid transport, protein metabolism, and both gene and cellular redox regulation [30]. Animal, and indeed human studies alike, have shown that 95% of luminal glutamate and 70% of glutamine, but only 40% of AKG, is metabolized (first pass) to CO_2 by the intestinal mucosa [11, 13]. Deamination of amino acids releasing amino nitrogen for growth results in the production of ammonia, which is generally protonated to ammonium at physiological pH. There is, however, some evidence that the ammonia ion itself may be an essential part of the appropriate environment for growth of enterocytes [32]. Indeed, ammonia, which is toxic and therefore maintained at a very low level in the blood, has been shown to be converted from extracellular or intra-mitochondrially generated ammonia to urea by enterocytes in post-weaned pigs [30]. Alternatively, AKG, which serves as a natural scavenger of ammonium, facilitates its conversion to amino acids and protein [21, 25, 31], and by reducing levels of ammonium in the body it has a beneficial effect on nitrogen metabolism. Indeed, in this way, one might also regard AKG as having a role as a protective agent for kidney function [29], with a knock-on benefit for osteoporosis, which is one of the most common bone disorder linked with nutritional and environmental factors [6, 7, 8].

Whilst the effects of PHA on intestinal cell turnover and those of AKG on nitrogen metabolism have been studied independently, their combined effect remains unknown. One might, however, suppose that an accelerated cellular turnover in enterocytes upon addition of PHA, resulting in an increase in ammonia production, in the presence of AKG, will rather promote its incorporation into amino acids and reduce the levels of urea produced, thereby alleviating renal function while at the same time enhancing amino nitrogen levels for growth.

The aim of the present study was therefore to investigate the effect of oral supplementation of PHA alone and in combination with AKG on GI-tract morphology and the growth and nitrogen balance in rats.

METHODS AND MATERIALS

Rats came from the Sprague Dawley outbred Laboratory (M&B A/S, Denmark), and were caged individually in metabolic cages (60% humidity with 12 h of light per 24 h) at the Dept. of Animal Physiology, Lund, Sweden. Rats were given *ad libitum* access to food: a wheat, barley, extracted soya bean meal, fat blend, mineral, vitamin and amino acid mix with a dietary composition (%) Dig. Crude Protein 17.37, Dig. Crude Opil 3.76, Calcium 0.65, total Phosphorus 0.51 of which 0.24 was available, Lysine 1.26 and Methionine 0.30, and a Dig. Energy of 13.75 MJ/kg.

Table 1. Composition of diet fed to rats for 11 days (Experiment 1) and 9 days (Experiment 2). Main ingredients: wheat, barley, extracted soya bean meal, fat blend, minerals, vitamin premixa, amino acids.

Calculated dietary composition	%
Dig. Crude Protein	17.37
Dig. Crude Oil	3.76
Dig. energy, MJ/kg	13.75
Calcium	0.65
Total Phosphorus	0.51
Available Phosphorus	0.24
Lysine	1.26
Methionine	0.30

[a]Providing the following per kilogram of diet (g/kg diet): Vitamin A, 14960 I.U; Vitamin D_3, 1574 I.U; Vitamin E, 110.4 mg; Thiamin, 17.6 mg; Riboflavin, 13.2 mg; Pyrodoxine, 17.9 mg; Vitamin B12, 35 µg; Vitamin K, 19.9 mg; Folic Acid, 3.1 mg; Nicotinic Acid; 34.1 mg; Panthotenic Acid, 34.1 mg; Choline, 1557.0 mg; Inositol, 1811.4 mg; Biotin, 369.2 µg.

The vitamin content per kg of diet comprised: Vitamin A, 14960 I.U.; Vitamin D_3, 1574 I.U.; Vitamin E, 110.4 mg; Thiamin, 17.6 mg; Riboflavin, 13.2 mg; Pyrodoxine, 17.9 mg; Vitamin B12, 35 µg; Vitamin K, 19.9 mg; Folic Acid, 3.1 mg; Nicotinic Acid; 34.1 mg; Panthotenic Acid, 34.1 mg; Choline, 1557.0 mg; Inositol, 1811.4 mg; Biotin, 369.2 µg (Tab. 1). The study was approved by the Ethical Review Committee for Animal Experiments at Lund University, and conducted according to European Community regulations concerning the protection of experimental animals.

Groups were equalised with regard to body weight. Food and water consumption as well as urine and faeces production were measured daily. Treatments and experimental conditions were conducted according to the recommendations of the Federation of the European Laboratory Animal Science Association (FELASA).

Experiment 1. Rats were assigned to 2 experimental groups and kept in metabolic cages: 1) Control, 2) PHA, with 6 animals in the Control group, and 6 in the treatment group. Stomach tube was applied once each day in the morning in all animals, while PHA was administered via a stomach tube in PHA group. The stock solution of crude PHA in 0.9% NaCl was (20% w/v) in water: 50 mg PHA/ml, 20 ml/kg body weight. Control rats were administered HCl (1 M) orally at a rate of 2.1 ml/l in the drinking water to compensate for the acidic effect of the PHA preparation (pH 6.0). Male rats were 17 weeks old at the start of the trial and remained part of the study for 11 days. Their initial body weight was 236.0 ± 1.4 g.

Experiment 2. Male rats were divided into 3 experimental groups and kept in metabolic cages: 1) Control, 2) AKG, 3) AKG+PHA, with 5 animals in the Control group and 6 in the treatment groups. Stomach tube was applied

once each day in the morning in all animals, while PHA was administered *via* a stomach tube in PHA group. AKG was administered *via* the drinking water. The stock solution of crude PHA in 0.9% NaCl was administered (20% w/v) in water: 50 mg PHA/ml, 20 ml/kg body weight. Control rats were administered HCl (1 M) orally at a rate of 10.5 ml/l in the drinking water to compensate for the acidic effect of the AKG preparation (pH 2.0). Rats were 17 weeks old at the start of the trial and remained part of the study for 9 days. Their initial body weight was 252.6 ± 1.0 g.

Urine and faeces sampling. Collection of urine into a cup was performed every day, with 10 ml of a 50% H_2SO_4 solution being used to stop microbial activity, while faeces, which was also collected in a cup, was kept dry. The total volume of urine was measured, and a homogenised sample of 10 ml was collected for urea analysis. The weight of the faeces was also measured on a daily basis, followed by collecting into 60 ml of a 50% H_2SO_4 solution. At the end of the experiment the total collection of faeces was analysed for nitrogen content.

Analysis of total Nitrogen. Nitrogen content was measured on a Leco Nitrogen and Protein Determinator FP-428 (Leco Corporation. St. Joseph. MI., USA). The instrument was set up in low range mode. Prior to analysis, the faecal samples were homogenised (Sorvall Omni-Mixer). The faecal samples, 150–200 mg, were analysed in tin foil cups. The urine samples (100–200 µl) were analysed in tin capsules measuring 15 × 6 mm (length/diameter). To obtain urine samples for nitrogen determination, aliquots of 1% of the daily urine volume were pooled. A glycine standard solution containing 1% nitrogen was used as a reference. All nitrogen determinations were made on duplicate samples.

Collection of GI-tract content and samples for histopathological analysis. At the end of the trial, rats were euthanized with an overdose of pentobarbital; stomach contents, proximal and distal small intestine, caecum and colon were collected, weighed and prepared for measurement of AKG concentration. Duodenum was fixed in Bouin's solution, and then dehydrated and embedded in paraffin. Paraffin sections (5 µm) were cut and mounted on Super Frost/Plus slides (Histolab, Sweden). For histological study using a light microscope, a hematoxylin and eosin stain was used. Duodenal preparations were measured for crypt depth, tunica mucosa thickness, epithelial cell height and basal width. From each animal, 40 well-oriented villi and crypts not taken from an area with Peyer's patches, were measured at low magnification (objective × 2.5). Tunica mucosa thickness was measured as the distance from the muscularis mucosa to the tip of villus, whereas crypt depth was measured from the muscularis mucosa to the level of the crypt opening. The enterocyte height of 10 well-oriented villi was measured using high magnification (objective × 100). One thousand cells were examined from each animal.

Immunohistochemistry. Immunohistochemical reactions were performed with monoclonal antisera against neuropeptide Y (Sigma, Sweden) or cholecystokinin (CCK) (Sigma, Sweden) using a dilution of 1/8,000. An indirect avidin-biotin-peroxidase method was applied, using Histomouse SP Bulk kit Broad Spectrum (Zymed, USA). Peroxidase was visualised using 3-amino, 9-ethylcarbazole as a chromogen, and the sections were lightly counterstained with hematoxylin according to the manufacturer's instructions. Sections were stained at the same time. The specificity of the stain was controlled by substitution of the primary antibody for buffer.

Calculations and statistics. Total feed intake was calculated from the level of food used per day, although an approximation was made to compensate for any feed which entered the cup used for collecting feces. This correction factor was found to be constant for all treatments and amounted to 10.77% of the total feed provided. The final feed intake was therefore adjusted using this factor. The nitrogen level of the feed was calculated from the level of protein in the feed – 19.76%. Data, which were normally distributed and of equal variance, were analysed for statistical significance between means using the student's t-test. A comparison of each variable in the histopathological analysis was performed using a one-way ANOVA. If the overall ANOVA proved to be significant, pair-wise comparisons using a t-test were then performed. In all statistical analyses $p<0.05$ was considered significant.

RESULTS

Performance. Experiment 1: the average starting weight of the Control group, was 237.6 g, and the body weight of the treated groups was not significantly different from that of the Controls. The results showed significantly lower daily weight gain as well as final body weight (data not shown) for the PHA group, compared to the Controls ($p<0.05$) (Tab. 2). Compared to the Control group, feed intake was significantly reduced in the PHA group (approx. 10% reduction; $p<0.05$) (Tab. 2).

Experiment 2: the average starting weight of the Control group was 252.6 g, and the body weight of the treated groups was not significantly different from that of the Controls. The results showed a significantly lower daily weight gain in the AKG+PHA group ($p<0.05$), compared to that of the Control and AKG groups (Tab. 2). The final body weight was also found to be lighter compared to both the Control and AKG groups ($p<0.05$, data not shown). Total water intake appeared reduced in the treatment groups compared to the Controls; this difference, however, was only significant for the AKG+PHA group (47% lower; $p<0.05$).

Urinary and faecal N. In experiment 1, a significant reduction in urine production was found between the Controls and the PHA treated rats, although no difference in the

Table 2. Mean daily growth performance, feed and water intake, excretion of urine and feces in rats.

Measurements	Experiment 1		Experiment 2		AKG+PHA
	Control	PHA	Control	AKG	
Daily gain (g)	4.8 ± 0.3	2.9 ± 0.9*	5.4 ± 0.4	6.0 ± 0.4	3.2 ± 0.3*
Feed intake (g)[a]	22.9 ± 1.9	20.5 ± 1.6*	22.4 ± 1.2	23.6 ± 0.8	19.8 ± 1.0
Water intake (mL)	53.3 ± 9.5	21.2 ± 2.6*	48.0± 6.0	33.4 ± 2.2	25.4 ± 3.4**
Urine (Ml)	33.1 ± 7.1	8.9 ± 1.5*	31.9 ± 6.2	16.1 ± 1.7*	10.7 ± 1.7*
Feces (g)	11.2 ± 0.6	11.9 ± 0.5	11.7 ± 2.1	11.6 ± 1.5	11.4 ± 2.9

Values are means ± SEM. Significance of changes against Control in each experiment (Student's t-test): *p <0.05, **p < 0.01. [a]Correction was made to compensate for any feed which entered the cup used for collecting feces (≈10.77% of total feed provided).

Table 3. Mean daily intake and excretion of nitrogen (N) in rats.

Measurements	Experiment 1		Experiment 2		AKG+PHA
	Control	PHA	Control	AKG	
Intake of N in feed (g/d)[a]	0.65 ± 0.02	0.58 ± 0.04	0.68 ± 0.03	0.72 ± 0.02	0.61 ± 0.03
N in Urine (g/d)	0.36 ± 0.02	0.30 ± 0.02*	0.38 ± 0.02	0.33 ± 0.02	0.25 ± 0.01**
N in Feces (g/d)	0.13 ± 0.003	0.14 ± 0.01	0.14 ± 0.007	0.15 ± 0.01	0.15 ± 0.01
N for Growth (g)[b]	0.12 ± 0.01	0.07 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.08 ± 0.006
N balance (g)	0.16	0.14	0.16	0.24	0.21
Urine N/ intake N (%)	55.4	51.7	55.9	45.8	40.9
Fecal N/ intake N %	20.0	24.1	20.6	20.8	24.6
Fecal N/ (urine N + fecal N) (%)	26.5	31.8	26.9	31.2	37.5
Relative consumed protein in urine vs feces (%)[c]	56.6 : 25.1	52.5 : 24.4	60.9 : 22.3	50.3 : 22.7	45.5 : 27.1

Values are means ± SEM. Significance of changes against Control in each experiment (Student's t-test): *p<0.05, **p<0.001. [a]Calculated level of consumed N = (Total feed consumption × protein level in feed (0.1967) × true protein dig. (0.868))/6.25. [b]Calculated level of N used for growth = (Total growth, g) × level of protein in flesh (0.16))/6.25. [c]Protein content was estimated by multiplying total N × 6.25 (based on FAO/WHO, 1973).

total level of excreted faeces was found between the two groups (Tab. 2).

In Experiment 2, there was a significant reduction in the level of urine excreted in both the AKG and AKG+PHA groups compared with the Controls ($p<0.05$), with the combined effect of AKG+PHA yielding a greater reduction than that of AKG alone – 66% and 50%, respectively. There was no difference between treatments with respect to the final amount of faeces excreted after 9 days of trial ($p<0.05$) (Tab. 2).

In Experiment 1, a significant reduction in N excretion in urine was observed in the PHA group, compared to the Control group (16% lower; $p<0.05$) (Tab. 3). Similarly, in Experiment 2, a reduction in N excretion in urine was observed in the AKG+PHA group, compared to the Control and AKG groups (34% lower; $p<0.05$). However, in the AKG group numeric reduction (trend) of N excretion was observed, compared to Control.

Calculation of the relative level of N in faeces to the total N intake (Faecal N/intake N%) showed that in AKG+PHA treated animals the percentage of N excreted in faeces increased, compared to the Control group, in favour of N excretion via the faeces. This was also visible after calculating the percentage of N excreted in faeces in relation to the N excreted in both faeces and urine (Faecal N/(urine N + faecal N) %) (Tab. 3).

Similarly, after hypothetical estimation of ingested protein, in AKG+PHA and in AKG treated animals the percentage of proteins excreted in faeces and urine differed compared to the Control group in favour of excretion via the feces (Tab. 3).

Gastro-intestinal tract weights. Experiment 1: In the PHA group, an increased total GI-tract weight was observed, compared to that of the Control group ($p<0.05$) (Tab. 4).

Experiment 2: In general, the changes influenced by the different treatments were related to the small intestine and large intestine. In the AKG+PHA group an increased weight of both small intestine and large intestine was noted ($p<0.05$) (Tab. 4).

Gastro-intestinal tract morphology. Experiment 2: The morphometric analysis of the duodenum taken from animals treated with PHA+AKG showed a significant difference ($p<0.05$), compared to that of the Control group with regard to crypt depth (138% increase compared with the 173.9 ± 18.7 μm crypt depth of the Controls. No significant difference in the thickness of tunica mucosa was found between the Control and treatment groups. Moreover, morphometric analysis of the intestinal cells – height and basal width of enterocytes – showed no difference

Table 4. Mean weights (g) of the selected rat GI-tract parts at the end of the experiments.

GI-tract	Experiment 1		Experiment 2		AKG+PHA
	Control	PHA	Control	AKG	
Stomach (g)	1.2 ± 0.03	1.2 ± 0.05	1.5 ± 0.07	1.4 ± 0.07	1.4 ± 0.04
Small Intestine (g)	6.6 ± 0.13	7.9 ± 0.52	6.9 ± 0.06	7.1 ± 0.33	8.1 ± 0.30**
Large Intestine (g)	1.1 ± 0.13	1.0 ± 0.02	1.2 ± 0.07	1.3 ± 0.08	1.5 ± 0.06*
Cecum (g)	1.1 ± 0.13	1.2 ± 0.08*	1.3 ± 0.1	1.2 ± 0.07	1.4 ± 0.08
Total GI-tract (g)	10.0 ± 0.11	11.3 ± 0.63*	10.9 ± 0.09	11.0 ± 0.38	12.4 ± 0.38*

Values are means ± SEM. Significance of changes against Control in each experiment (Student's t-test): *p<0.05, **p<0.01.

between the Control group and the AKG+PHA treated rats. (data not shown).

The results of immunohistochemical examination of the duodenum showed no difference in abundance of CCK positive cells in the tunica mucosa between Controls and the AKG+PHA group. However, a difference in neuropeptide Y (NPY) positive material was noticeable. In the Control group, the expression of NPY was present in both granules of neuronal cells of the submucosal parasympathetic ganglia and goblet cells of the epithelium. In contrast, in the AKG+PHA group, only expression of NPY in granules of neuronal cells of the submucosal parasympathetic ganglia, without any expression in goblet cells, was noticeable (data not shown).

DISCUSSION

Effect of PHA and AKG on urinary and faecal N balance. Daily urine excretion depends on a number of different factors, among which water intake is one of the most important, yet under physiological conditions it has no influence on total urea (and nitrogen) excretion *via* the kidneys [22]. The present study shows that AKG+PHA treatment alters the proportion of N excreted in urine *cf* that of faeces, so that faecal N excretion appears to be favoured. Moreover, it is important to note that the addition of AKG alone to the diet did not exert any influence on the amount of N excreted in the urine or faeces. The reduction of the urine excretion in PHA treated rats was not followed by the reduction of N excretion via urine and therefore, it may be attributed to the reduced water intake.

Rat performance. In the present study, PHA treatment in rats negatively influenced both performance and food intake, which supports previous findings showing that the ingestion of PHA (from red kidney beans) leads to a reduction in body weight, and at high doses may damage the gut mucosa. However, lectin at low doses (0.01–0.2 g/kg BW) does not exert a strong anti-nutritional effect in rats and, in general, may cause a reversible dose-dependent hyperplastic growth of the intestinal wall [2]. Moreover, results from a recent piglet study showed an improvement in performance with PHA treatment [27]. This positive effect on performance was attributed to a lower total diarrhoeal score, which in itself can arise through an increased maturation of the gastro-intestinal (GI) tract post-weaning, with PHA treatment [20]. In the present study we found that PHA treatment had no adverse effect on GI-tract weights in terms of stomach, small and large intestine and caecum. In fact, there was a trend towards an increase in total GI-tract weight (113% with PHA *cf* Controls), although this was not significant. Similarly, AKG treatment had no adverse effect on GI-tract weights. Interestingly, the addition of AKG+PHA to the diet significantly increased small intestinal weight by 117% and large intestine weight by 125% *cf* Controls. The results of the present study pertaining to the weight of the GI-tract generally substantiate previous findings [1, 2, 16, 18, 22] in that the weight of the small intestine was significantly increased with PHA treatment. This was also confirmed by calculation of the ratio of total body weight/GI-tract weight, where in the PHA treatment group the ratio was 4.5 (AKG+PHA), while in the Control and AKG treatment groups the ratio was 3.6 (data not shown).

Effect of PHA and AKG on the morphology of the gut & CCK/NPY expression. Increased crypt depth in PHA treated animals supports the findings of previous studies [2, 14, 16, 20] in that PHA treatment increases the weight of the small intestine by increasing cell production rate. The underlying mechanism for the PHA effect is most likely the same as for some peptide growth factors and hormones – it binds to cell surface receptors of the brush border membrane, and acts as an extraneous growth factor in the gut. By interacting with brush border epithelial receptors, PHA induces extensive proliferation and changes in the metabolism of epithelial cells by activation of second messenger pathways [2]. It has also been shown that plant lectins bind avidly to the mucosal surface and induce dose- and time-dependent as well as fully reversible hyperplastic and hypertrophic growth in the small intestine [2].

PHA is one of the most powerful growth factors in the rat alimentary tract [1, 3]. A 10-day oral PHA treatment induces dose dependent growth in the small intestine and pancreas. However, the mechanism underlying this effect remains unknown [18]. Gastro-intestinal peptides, especially cholecystokinin (CCK), have been shown to be involved in the regulation of pancreas growth and function [17]. It has also been shown that PHA significantly stimulates small

intestinal growth by a CCK independent mechanism, and pancreatic hypertrophy by a CCK dependent mechanism [10, 24]. Moreover, PHA *in vitro* directly and dose dependently releases CCK from isolated intestinal mucosa cells [5]. The exact mechanism of lectin-induced CCK release from CCK cells in the intestine has yet to be elucidated, but it is hypothesised that lectins influence CCK cell activity by binding to the glycosyl side chains of voltage-gated channels [23]. Among other peptides and hormones (e.g. gastrin, enteroglucagon, glucagon, and peptide YY) which have been profoundly investigated under PHA treatment, only insulin has been shown to have a trophic effect on the pancreas and the intestine of rats [1, 4]. NPY is a peptide that is known to stimulate feeding, and NPY – immuno-reactive nerve cells are ubiquitous in the submucosal ganglia. Our results indicate that oral PHA+AKG treatment may lead to an alteration of NPY expression in the duodenum of rats. The physiological effect of this alteration may be an increase in duodenal intra-luminal pressure, together with involvement in the regulation of inter-digestive motility of the small intestine [9]. It may therefore be speculated that AKG+PHA, instead of influencing gut morphology, may rather exert an effect on the rapidity of feed passage through the GI-tract of an animal, the so-called transit time.

In conclusion, the present results indicate that PHA, administered alone or in the presence of AKG, stimulates small bowel growth in rats by increasing crypt depth and the weight of the small intestine. The consequence of such changes will most likely be: 1) a higher rate of protein production in the intestinal wall, and 2) a change in the proportion of N excretion via urine and faeces, apparently favouring faecal excretion. While it is proposed that further studies are needed in order to elucidate the action and role of AKG, this study reveals new perspectives for the treatment of injured intestinal mucosa, as well as some potential in prolonging the inter-dialysis period in subjects with chronic renal failure.

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